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CELL MULTIPLICATION IN THE SUB-CUTICULA OF DILEPIS SCOLECINA.¹

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INTRODUCTION.

The process of cell division in cestodes as compared with that in other Metazoa is apparently quite abnormal. An examination of cestode material at once reveals the fact that mitotic figures are very rare, and that an explanation of the process of cell division analogous to any of the common types is apparently impossible. The opinion of the various workers in cestode cytology, as to how cell division is taking place, varies greatly. Some state that it occurs by mitosis, others by amitosis, while it has been asserted that nuclei arise 'de novo' from the cytoplasm.

Child ('07) noted the apparent infrequency or total absence of any evidence of mitosis in *Moniezia*, even in regions where rapid growth was taking place. He says, "If my observations are correct, amitosis is the more common method of division in the generative cycle, except during the period of maturation and early cleavage. And in the somatic cells of the adult body it appears to be the usual method at all times."

Young ('08), working with *Cysticercus pisiformis* describes what he calls the "de novo" formation of cells. He observed irregular masses of coarsely granular cytoplasm lying in the meshes of the parenchyma network. These masses contain numerous small deep staining granules scattered haphazard through the mass. Shortly succeeding the formation of these granules, a nuclear membrane is formed around them; the newly formed nucleus, together with a small mass of cytoplasm, becomes partly constricted from the parent mass; and the daughter cell has been formed."

Further, he says: "I believe that the nucleus in these forms is not a morphological, but a physiological entity; that the

¹ A thesis presented to the graduate faculty of the University of North Dakota in partial fulfilment of the requirements for a master's degree.

nuclear granules are fundamentally the same as the remaining protoplasm of the cell, but are differentiated therefrom under physiological conditions which we do not at present understand; that the granules are perhaps reserve material stored up in the nucleus for future use, the entire cell body being thus occasionally converted into a nucleus; and the nucleus varies in structure from time to time in response to the varying physiological demands made upon it. . . . Further if my interpretation of my observations be correct, then distinction between germ and somatic plasm is obviously impossible, a special vehicle for the transference of hereditary qualities is entirely wanting; such qualities must be transmitted by the undifferentiated protoplasm; cell lineage is manifestly lacking; a mosaic theory is plainly untenable; and the fate of any given embryonic element—whether it shall form parenchyma, muscle, nerve, etc.—must be determined by physiological causes alone."

Richards (1911), working with *Moniezia*, does not agree with Child. He says (p. 158): "I have after diligent search upon carefully prepared material been unable to establish a series of stages in the autoconstriction and subsequent division of the nucleus and cell body by amitosis. Considering the evidence as set forth, it seems to the writer that one is forced to the conclusion that mitosis is the method by which pre-oögonia and cleavage divisions are accomplished."

Mary T. Harman ('13, p. 223) states: "My observations have not shown that amitosis does not take place in *Taenia* or *Moniezia*, but they have shown no condition which cannot be as readily explained as the result of mitotic as of amitotic division."

MATERIALS AND PROCEDURE.

The form I worked with was *Dilepis scolecina* parasitic in the small intestine of the double-crested cormorant (*Phalacrocorax dilophus*). These birds are found abundantly near the shores and on the islands of Devils Lake, North Dakota.

Immediately after the bird was killed, the cestodes were removed from the intestine and placed in fixing solution. Flemming's solution and cestode mixture were the fixatives used. Flemming's solution blackened the tissue so that the results

from it were not satisfactory. The cestode mixture, however, gave excellent results.

The stains used were the following: Heidenhain's iron-alum-hæmatoxylin without counterstain; safranin counterstained with light green; thionin counterstained with acid fuchsin; methyl green counterstained with acid fuchsin; and safranin counterstained with water blue.

OBSERVATIONS.

I began my study of cell multiplication in cestodes without any previous knowledge of what had been done in the field of cestode cytology. Moreover, I completed the study of my material and drew my conclusions before I read any of the literature on the subject.

I have confined my study of cell multiplication in *Dilepis* to the sub-cuticula. In this tissue I have searched in vain for a single clear case of mitosis or amitosis. Moreover, in order to be certain I had not overlooked any, I counted 10,000 resting nuclei in the sub-cuticula of the neck regions of ten worms with the same result. Certainly active growth must have been taking place in this region, but it could not be accounted for by mitotic or amitotic division.

I have, however, observed numerous places in this region in which active cell multiplication was apparently taking place. Here multinucleate cells, such as shown in Fig. 1, have been observed. In addition to these, large protoplasmic masses were present, which varied in size from that of a single cell to that of perhaps fifty cells massed together. Fig. 2 shows a typical mass. These masses stain rather deeply with nuclear stains, and contain from one to five nuclei.

These masses are found abundantly in the neck region of every worm I examined, and occur, although less frequently, in the body region.

By reference to any of these figures it is seen at once that the mass of cytoplasm is out of proportion to the mass of the nuclei. Moreover, I have observed numerous lobes and occasionally even entire masses in which I was unable to find any trace of a distinct nucleus. Fig. 7 shows a lobe,¹ *i*, and Fig. 6 a mass of

¹ At focal levels other than that shown in the figure the lobe was seen to be continuous with nucleate masses.

protoplasm, *h*, in which no well-defined nucleus is present. However, in this latter case the mass is so close to a nucleate mass that I cannot say positively that it is not continuous with it.

By closely examining the nuclei present in these masses, I find that the nuclear membranes are very indistinct in many cases. Fig. 2 shows a mass in which the nuclei have indistinct membranes. Also one of the nuclei, *c*, has a somewhat less distinct membrane than the other, *b*. And this latter membrane is in turn less distinct than the membranes of the nuclei in the cell syncytium above it.

Moreover, a large number of nuclei have been seen which lack membranes completely. The nucleus consisted of a "nucleolus" or "karyosome" surrounded by a clear zone. Figs. 3, 4, and 5 show "karyosomes" which lack membranes. As Child and Young have already suggested, I believe this "nucleolus" represents the chromatin material of the nucleus.

By observing the protoplasm under high magnification (2,000 diameters) it is seen that the protoplasmic strands contain many dark staining granules of various sizes and shapes. Some of these granules were as large as the "nucleoli" of the complete nuclei; others, however, were so small as to be scarcely discernible. Fig. 4 shows a mass which contains a number of varying-sized granules. Fig. 5 shows a mass which contains a number of varying-sized granules one of which, *g*, is becoming surrounded by a clear zone.

The protoplasmic masses apparently arise by the outgrowth of protoplasm from certain cells of the syncytium. Figs. 2, 3, 4, and 6, show masses of protoplasm continuous with the syncytial cells around them. In Fig. 6, the developing mass is very small and contains no definite nucleus. In Figs. 2, 3, and 4, the masses are very large and contain from one to five complete nuclei. A large number of masses have been observed varying in size between these extremes. The nuclear membranes of the nuclei in the cells from which these masses are developing, contain very small, irregular granules which stain darkly like the granules in the cytoplasm. I have insufficient evidence for or against Young's view of the "de novo" origin of these granules. The chromatin granules may arise "de novo" in the cytoplasm and

develop to complete nuclei in situ. Young bases his theory of the independent origin of granules from a cytogenic protoplasmic mass upon the following facts:

1. The occurrence of masses of granular protoplasm lacking any evident nuclei.

2. The occurrence of isolated "nucleoli" of varying size from $\frac{1}{4}$ to 1 micron in diameter, which are usually found in the above mentioned masses of protoplasm but occasionally lie free in the parenchyma strands.

I believe, however, that these facts may be equally well accounted for by assuming the extrusion of chromidia from a mother nucleus. Masses of granular protoplasm without any evident nuclei, which occur but rarely may be explained as having been severed from parent masses after impregnation with chromidia. The occurrence of isolated "nucleoli" can be accounted for just as well by assuming the migration of chromidia from the nuclei along the strands of the cytoplasmic network, as by the assumption of their development from the protoplasm in situ.

Young, in a later paper ('13) dealing with gametogenesis, in *Tænia pisiformis* says (p. 375): "I believe that new nuclei arise either from chromidial extrusions from old nuclei, or 'de novo' in the cytoplasm. . . . The structure of the nucleus—a loose collection of chromatin bodies without a membrane—renders the extrusion of chromidia an easy matter. After their extrusion new chromatin is added and that part of the cell containing them is constricted off, to give rise in its turn to other cells. . . . It is obviously impossible to say, however, whether any chromatin granule in the cytoplasm is a chromidial extrusion or a 'de novo' formation."

Since I have seen these very small granules, all of about the same size, present in the nuclear membrane as though impeded by it in their exit, along the strands of the protoplasmic network, from the nucleus to the cytoplasm, I believe that these granules are extruded from the mother nucleus. Moreover, since I have observed granules of various shapes and sizes, many of the larger ones appearing to be composed of three or four smaller ones partly united, and since I have often seen a number of

granules clustered together, I believe that the larger granules are the result of the union of many smaller ones. Thus, I believe that the small particles of chromatin or "chromidia" are extruded from the mother nucleus. Then these "chromidia" unite here and there throughout the protoplasm to form larger granules or "karyosomes" which become surrounded by a clear zone. Finally the nuclear membrane is formed, producing a daughter nucleus. When a number of nuclei have been formed multinucleate cells are the result. Since the tissue is always a cell syncytium, constrictions of the cytoplasm around a nucleus finish the production of a daughter cell. Thus one mother cell may produce a large number of daughter cells.

COMPARISON WITH *TÆNIA PISIFORMIS*.

In order to compare the process of cell multiplication in *Dilepis* with that in other cestodes, Dr. Young has permitted me to examine his slides of *Tænia pisiformis*, and *Cysticercus pisiformis*. Here I have identified the protoplasmic masses in both the adult and the larva. These also contain nuclei in the various stages of formation from chromidia to complete nuclei. The young larvæ show large numbers of protoplasmic masses developing in the cell syncytium. In the older larvæ the masses often show four or five nuclei developing membranes at the same time.

DISCUSSION.

Cell multiplication by means of protoplasmic masses and the development of nuclei from chromidia, has, so far as I am aware, never been observed heretofore in Metazoa by anyone except Young. He has described the process as it occurs in *Cysticercus pisiformis* (Young, '08) and has noted it in some other cestodes (Young, '10) although his interpretation varies slightly from my own. I have, in the present paper given an account of it as it occurs in the sub-cuticula of *Dilepis scolecina*. It is true that chromidia have been observed in certain Metazoa, but no account of their functioning in the reproduction of the cell has ever been given previous to Young's paper on the "Histogenesis of *Cysticercus pisiformis*."

If cells are actually developing from protoplasmic masses in

the manner described, we have here an exceptional method of cell multiplication, unlike anything previously described in Metazoa.¹ Moreover, if future research supports this view, the present theories of the role of the nucleus in heredity will have to be greatly modified at least with respect to cestodes.

As Young has previously suggested, the explanation of such a method of cell multiplication as this may rest on the fact that the cestode is highly degenerate in most characteristics due to its long period of parasitism. In the development of cells from protoplasmic masses the nucleus passes through a cycle in which occur stages resembling nuclei of lower forms. The protoplasmic mass with its diffused nuclei in the form of chromidia is comparable to a cell of the Bacteria or of the Myxophyceæ. In certain Protozoa also, as noted by many observers, the nuclear material at certain periods diffuses throughout the cytoplasm in the form of chromidia which may give origin to secondary nuclei, and these in turn to gametes. It is possible that the cestode nucleus has lost the power of mitotic division, accompanying the somatic degeneration of the worm due to parasitism. Richards, Harman, and others have shown, however, that we still find cell division taking place by mitosis in the sex cells and developing embryos.

CONCLUSIONS.

I have made the following conclusions in regard to cell multiplication in the sub-cuticula of *Dilepis scolecina*.

1. After a careful examination, and after counting 10,000 of the nuclei in this region, I conclude that the growth of the sub-cuticula cannot be accounted for by mitotic or amitotic division.

2. Tissue growth is taking place rapidly in this region by the development of protoplasmic masses. My reasons for believing this are the following:

- A. The nuclei in the multinucleate cells are frequently seen crowded together as if they had developed in protoplasmic masses.

- B. In the protoplasmic masses the quantity of cytoplasm is out of proportion to the number of complete nuclei present.

- C. Developing nuclei have been actually observed in the cytoplasm. The different stages of nuclear formation are shown by the following:

¹ A similar process was suggested long ago by Schleiden and Schwann.

- (a) The chromidia, or diffused nucleus.
- (b) The irregular chromatin granules formed by the union of numerous chromidia and surrounded by a clear zone.
- (c) The nuclear membranes of the nuclei in the masses vary considerably from delicate, scarcely discernible membranes to heavy, well developed ones.
- D. These masses appear to arise by the simultaneous growth of cytoplasm and chromidial extrusions from the nuclei of certain cells.
- 3. The degenerate character of the nucleus is perhaps the result of the parasitic habit of the cestode.

I wish here to express my sincere thanks to Dr. R. T. Young, whose valuable criticisms and suggestions made this work possible. I also wish to express my indebtedness to Dr. B. H. Ransom for identifying my material.

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EXPLANATION OF PLATE.

- FIG. 1. Multinucleate cell, *a*.
FIG. 2. Nuclei with indistinct membranes, *b* and *c*.
FIG. 3. Nuclei, *d* and *e*, lacking nuclear membranes.
FIG. 4. Chromatin granules, *f*, in the cytoplasm.
FIG. 5. Large chromatin granule, *g*, in cytoplasm.
FIG. 6. A developing protoplasmic mass, *h*, in which no definite nucleus is present.
FIG. 7. A lobe, *i*, of a protoplasmic mass in which no definite nucleus is present.
FIG. 8. A large protoplasmic mass in the body region which contains only one nucleus, *j*.
FIG. 9. Protoplasmic masses, *k*, developing in the body region.

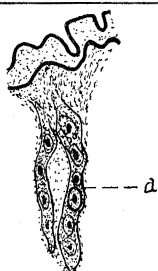


Fig. 1.

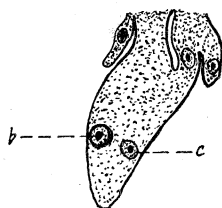


Fig. 2.

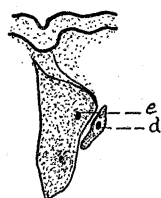


Fig. 3.

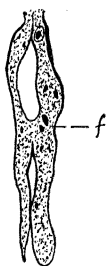


Fig. 4.

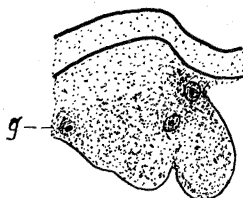


Fig. 5.



Fig. 6.

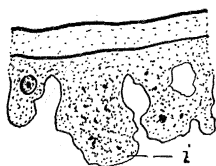


Fig. 7.

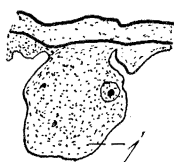


Fig. 8.

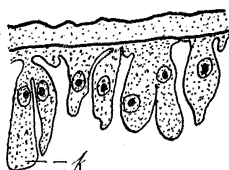


Fig. 9.